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TITLE: Mapping Mammary Epithelial Cell Transformation in BRCA1 Mutant Mice

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CONTRACTING ORGANIZATION: Beth Israel Deaconess Medical Center
Boston, MA 02215

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Mapping Mammary Epithelial Cell Transformation in BRCA1 Mutant Mice

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Beth Israel Deaconess Medical Center
Boston, MA 02215

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14. ABSTRACT

Recently developed culture models provide us with a window into the initial stages of breast cancer formation, when some molecular changes have already occurred but the typical malignant growth is not yet present. We have called these cells that have already taken one or more "hits", but do not yet form cancer, tumor progenitor cells. It is our hypothesis that the breast tissue of BRCA1 carriers harbors a population of cancer progenitor cells that can be identified with suitable culture techniques. We used a BRCA1 mutant mouse model to address this question. The BRCA1 mutant mice were bred and their breast tissue cells systematically analyzed in an assay that allows for the growth of these cells in three dimensions, where their growth pattern most closely resembles the mammary gland environment. Culture conditions for the growth of these mammary epithelial cells were established; i.e. growth in a 2% matrigel medium supplemented with EGF, Insulin and Hydrocortisone. Conditional BRCA1 mutant mice were bred together with the respective controls. Mammary glands were harvested at the ages of 6, 12 and 15 months. The differences in growth pattern of PMECs isolaed from these mice did not differ significantly.

15. SUBJECT TERMS
No subject terms provided.

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Introduction

Identifying early steps in the cancerous transformation of primary mammary epithelial cells (PMECs) may help guide treatment and prevention strategies. Female BRCA1 carriers are highly predisposed to developing breast cancer, and their normal breast epithelium has a high prevalence of pre-malignant lesions[1-3]. On the molecular level early genetic changes in histologically normal breast epithelium can precede morphological changes in the mammary gland in both, spontaneous, as well as in BRCA1 related breast cancers[2]. However, it is unclear which of these differentially expressed proteins are the key players in initiating or maintaining the malignant phenotype and how the genetic mutations lead to these changes. It is our hypothesis that the morphologically normal breast epithelium of BRCA1 carriers harbors tumor progenitor cells at various stages of malignant transformation, and that these cells have undergone some but not all of the genetic and gene expression changes necessary for malignant transformation. We proposed to test this hypothesis in a mouse model. We used an ex vivo colony formation approach to analyze single mammary epithelial cells from the normal breast epithelium of BRCA1-mutant mice (3) with the goals of 1. identifying tumor progenitor cells and 2. defining a map of the transformation of these breast epithelial cells. We chose the BRCA1 null mice as the closest model for human BRCA1 carriers. This ex vivo model may resemble in vivo conditions closely enough to allow for the experimental recapitulation of breast cancer formation.

Body

The initiation of breast cancer in humans is a complex process that is still largely unclear. Gene expression profile studies suggest that many, if not all, gene expression changes are already present at the level of atypical ductal hyperplasia, a pre-cancerous condition. Therefore, it was our aim to analyze the processes that proceed the formation of these pre-neoplastic changes as a thorough understanding of the initiation of breast cancer is pivotal for the development of chemoprevention and cancer treatment strategies. We have developed an in vitro system for the differentiation of primary mammary epithelial cells[4]. This system allows us to detect differences between the differentiation of normal versus mammary epithelial cells derived from oncogene carrying mice. We wanted to test whether these early differences in differentiation pattern were representative of early steps of carcinogenesis, and whether it allowed us to analyze the early steps of tumor formation.

For this purpose transgenic and conditional knock-out mice (mutant p53 or mutant BRCA1 mice and MMTV-Cre transgenic mice), have been bred, and their primary mammary epithelial cells analyzed before they develop tumors as well as in the tumors that do develop. The mice were bred, then genotyped. In general male newborns have been euthanized as this is a breast cancer study and the incidence of breast cancer in males is low. Only males that were kept for breeding purposes were raised. The females that are transgene positive, have been kept as virgins.

At various stages of mammary gland development they have been sacrificed and their mammary glands examined histologically and in the newly developed colony formation assay. These studies have been performed in the animals BEFORE they develop tumors to assess the genetic changes in a mammary epithelium "at risk" that has not yet undergone transformation. When females developed breast tumors, they were observed twice a week, and the observation has been documented in an Experimental Illness Form. Once the tumor reached a diameter of greater than 0.5 cm or if the tumor growth interferes with the mobility or the wellbeing of the mouse, the tumor-bearing mouse was sacrificed. In addition, if a tumor showed ulceration, the tumor-bearing mouse was sacrificed.

EXPERIMENT

FLOX+/+ J+ p53+/-	18 mice		
2055	FEMALE	(1656) J-Cre/FLOX+/+ X (2033) J-Cre/FLOX+/+/p-/-	4/28/2007
2056	FEMALE	(1656) J-Cre/FLOX+/+ X (2033) J-Cre/FLOX+/+/p-/-	4/28/2007
2057	FEMALE	(1656) J-Cre/FLOX+/+ X (2033) J-Cre/FLOX+/+/p-/-	4/28/2007
2059	FEMALE	(1665) J-Cre/FLOX+/+ X (2033) J-Cre/FLOX+/+/p-/-	4/28/2007
2060	FEMALE	(1665) J-Cre/FLOX+/+ X (2033) J-Cre/FLOX+/+/p-/-	4/28/2007
2061	FEMALE	(1656) J-Cre/FLOX+/+ X (2033) J-Cre/FLOX+/+/p-/-	4/28/2007
2062	FEMALE	(1656) J-Cre/FLOX+/+ X (2033) J-Cre/FLOX+/+/p-/-	4/28/2007
2032	FEMALE	(1804)J-Cre/FLOX+/+/p+/- X (1860)J-Cre/FLOX+/+/p+/-	2/14/2007
2037	FEMALE	(1861)J-Cre/FLOX+/+/p+/- X (1804)J-Cre FLOX+/+ p+/-	2/14/2007
2038	FEMALE	(1861)J-Cre/FLOX+/+/p+/- X (1804)J-Cre FLOX+/+ p+/-	2/14/2007
2101	FEMALE	(1656) J-Cre/FLOX+/+ X(2066) J-Cre/FLOX+/+p+/-	8/26/2007
2102	FEMALE	(1656) J-Cre/FLOX+/+ X(2066) J-Cre/FLOX+/+p+/-	8/26/2007
2107	FEMALE	(1655) J-Cre FLOX+/+ X (2063) J-Cre/FLOX+/+p+/-	8/26/2007
1809	FEMALE	(1517)J-Cre/FLOX+/-/p+/- X (1419)J-Cre/FLOX+/+	7/26/2006
1808	FEMALE	(1517)J-Cre/FLOX+/-/p+/- X (1419)J-Cre/FLOX+/+	7/26/2006
1858	FEMALE	(1819)J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	9/27/2006
2031	FEMALE	(1804)J-Cre/FLOX+/+/p+/- X (1860)J-Cre/FLOX+/+/p+/-	2/14/2007
2025	FEMALE	(1516)J-Cre/FLOX+/-/p+/- X (1803)J-Cre/FLOX+/-p+/-	2/8/2007

FLOX+/+ J+	18 mice		
1833	FEMALE	(1417/1244) J-Cre/FLOX+/- X (1409) J-Cre/FLOX+/+	8/7/2006
1689	FEMALE	(1418) J-Cre/FLOX+/- X (1409) J-Cre/FLOX+/+	7/31/2006
1692	FEMALE	(1418) J-Cre/FLOX+/- X (1409) J-Cre/FLOX+/+	7/31/2006
1493/1434	FEMALE	J-Cre/FLOX+/- (1241) x J-Cre/FLOX+/- (1239)	1/27/2006/
1851	FEMALE	J-Cre/FLOX+/+ (1493/1434) x (1659) FLOX+/+	9/15/2006
1848	FEMALE	J-Cre/FLOX+/+ (1493/1434) x (1659) FLOX+/+	9/15/2006
1849	FEMALE	J-Cre/FLOX+/+ (1493/1434) x (1659) FLOX+/+	9/15/2006
1868	FEMALE	(1492) J-Cre/FLOX+/+ X (1659) FLOX+/+	9/27/2006
1870	FEMALE	(1492) J-Cre/FLOX+/+ X (1659) FLOX+/+	9/27/2006
1871	FEMALE	(1492) J-Cre/FLOX+/+ X (1659) FLOX+/+	9/27/2006
1869	FEMALE	(1492) J-Cre/FLOX+/+ X (1659) FLOX+/+	9/27/2006
1872	FEMALE	(1492) J-Cre/FLOX+/+ X (1659) FLOX+/+	9/27/2006
1656	FEMALE	(1493) J-Cre/FLOX+/+ X (1409) J-Cre/FLOX+/+	7/6/2006
1862	FEMALE	(1492) J-Cre/FLOX+/+ X (1659) FLOX+/+	9/27/2006
1655	FEMALE	(1493)J-Cre/FLOX+/+ X (1409) J-Cre/FLOX+/+	7/6/2006
1657	FEMALE	(1493) J-Cre/FLOX+/+ X (1409) J-Cre/FLOX+/+	7/6/2006
1665	FEMALE	(1492) J-Cre/FLOX+/+ X (1409) J-Cre/FLOX+/+	7/6/2006
1493/1434	FEMALE	J-Cre/FLOX+/- X J-Cre/FLOX+/-	3/20/2006

FLOX+/+ J+ p53wt	14 mice		
2108	FEMALE	(1655) J-Cre FLOX+/+ X (2063) J-Cre/FLOX+/+p+/-	8/26/2007
2014	FEMALE	(1809)J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	10/25/2006
2015	FEMALE	(1809)J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	10/25/2006
2004	FEMALE	(1808) J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	10/23/2006
2005	FEMALE	(1808) J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	10/23/2006
2082	FEMALE		7/26/2007
1857	FEMALE	(1819)J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	9/27/2006
1854	FEMALE	(1819)J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	9/27/2006
1700	FEMALE	(1517)J-Cre/FLOX+/-/p+/- X (1419)J-Cre/FLOX+/+/p+/-	7/26/2006

1697	FEMALE	(1517)J-Cre/FLOX+/-/p+/- X (1419)J-Cre/FLOX+/+/p+/-	7/26/2006
1885	FEMALE	(1698) J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	10/6/2006
1882	FEMALE	(1698) J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	10/6/2006
1883	FEMALE	(1698) J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	10/6/2006
1898	FEMALE	(1679) FLOX+/+ X (1804) J-Cre/FLOX+/+/p+/-	10/25/2006
1899	FEMALE	(1679) FLOX+/+ X (1804) J-Cre/FLOX+/+/p+/-	10/25/2006

CONTROLS

NO FLOX — 3 mice

1852	FEMALE	J-Cre/FLOX+/+ (1493/1434) x (1659) FLOX+/+	9/15/2006
1875	FEMALE	(1244/1417) FLOX+/- X (1659) FLOX+/+	9/27/2006
1884	FEMALE	(1698) J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	10/6/2006

FLOX+/- J+ p53+/-

2024	FEMALE	(1516)J-Cre/FLOX+/-/p+/- X (1803)J-Cre/FLOX+/-p+/-	2/8/2007
1691	FEMALE	(1418) J-Cre/FLOX+/- X (1409) J-Cre/FLOX+/+	7/31/2006
2022	FEMALE	(1516)J-Cre/FLOX+/-/p+/- X (1803)J-Cre/FLOX+/-p+/-	2/8/2007
2044	FEMALE	(1816)J-Cre/FLOX-/-/p-/- X (1830)J-Cre/FLOX+/-/p+/+	3/24/2007
1821	FEMALE	(1517)J-Cre/FLOX+/-/p+/- X (1419)J-Cre/FLOX+/+/p+/-	7/26/2006
1824	FEMALE	(1516) J-Cre/FLOX+/-/p+/- X (1419) J-Cre/FLOX+/+/p+/-	8/7/2006
1822	FEMALE	(1516) J-Cre/FLOX+/-/p+/- X (1419) J-Cre/FLOX+/+/p+/-	8/7/2006

FLOX+/- J+ p53+/+ — 3 mice

1825	FEMALE	(1516) J-Cre/FLOX+/-/p+/- X (1419) J-Cre/FLOX+/+/p+/-	8/7/2006
1690	FEMALE	(1418) J-Cre/FLOX+/- X (1409) J-Cre/FLOX+/+	7/31/2006
1818	FEMALE	(1517)J-Cre/FLOX+/-/p+/- X (1419)J-Cre/FLOX+/+/p+/-	7/26/2006

FLOX+/- NO J-CRE p53 wt — 2 mice

1873	FEMALE	(1244/1417) FLOX+/- X (1659) FLOX+/+	9/27/2006
1876	FEMALE	(1244/1417) FLOX+/- X (1659) FLOX+/+	9/27/2006

FLOX+/+ NO J-CRE p53+/- — 2 mice

2058	FEMALE	(1656) J-Cre/FLOX+/+ X (2033) J-Cre/FLOX+/+/p-/-	4/28/2007
2103	FEMALE	(1656) J-Cre/FLOX+/+ X(2066) J-Cre/FLOX+/+p+/-	8/26/2007

CANCER MICE (FEMALES) — 8 mice

TAG	GENDER	PARENTS	DOB
1860	FEMALE	(1819)J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	9/27/2006
1858=2053	FEMALE	(1819)J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	9/27/2006
1819	FEMALE	(1517)J-Cre/FLOX+/-/p+/- X (1419)J-Cre/FLOX+/+	7/26/2006
1861	FEMALE	(1819)J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	9/27/2006
1515	FEMALE	(1220) p+/- X (1406) F+/+,J+	4/7/2006
1853	FEMALE	(1819)J-Cre/FLOX+/+/p+/- X (1659) FLOX+/+	9/27/2006
1897 (Tumor Mouse)	FEMALE	(1679) FLOX+/+ X (1804) J-Cre/FLOX+/+/p+/-	10/25/2006
1801	FEMALE	(1517)J-Cre/FLOX+/-/p+/- X (1419)J-Cre/FLOX+/+/p+/-	7/26/2006

The number of colonies derived from the primary mammary epithelium of these mice at the ages of 6 and 9 months was not significantly different. We did, however, observe that the diameter of the colonies was larger in the BRCA1 null PMECs. We are therefore currently examining these colonies with specialized image analysiis software. We have, as originally planned, created a tissue bank with fixed paraffin-embedded tissues. At this point we decided to pursue the project in two different ways: Compare BRCA1-null PMECs with controls at the age of 15 months, and, introduce a second mutation that is often found early-on in BRCA1-related cancers, p53 heterozygozity.

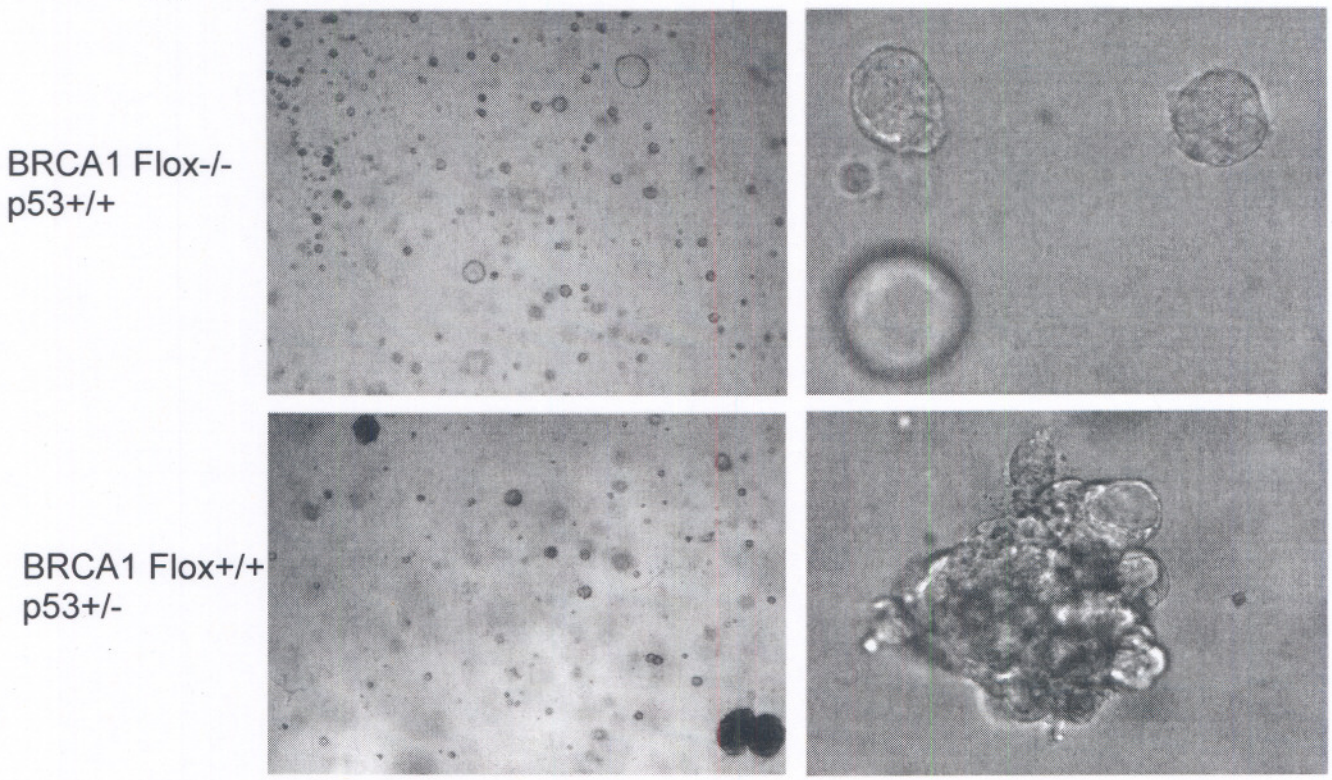
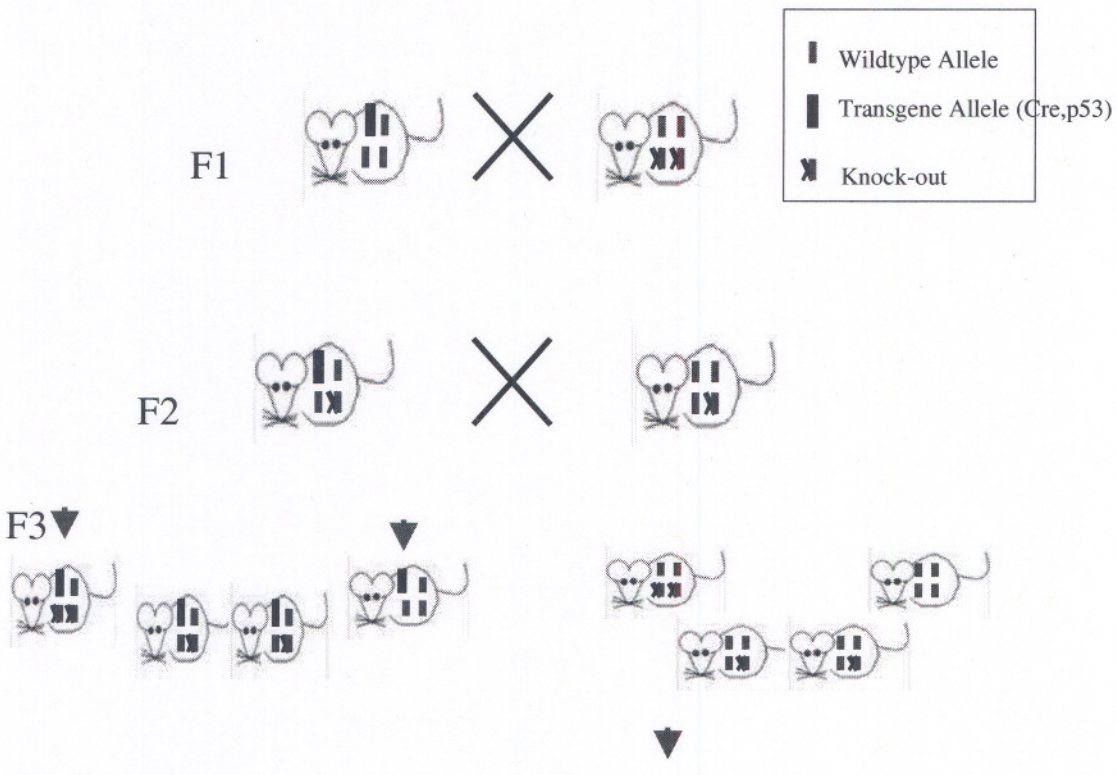


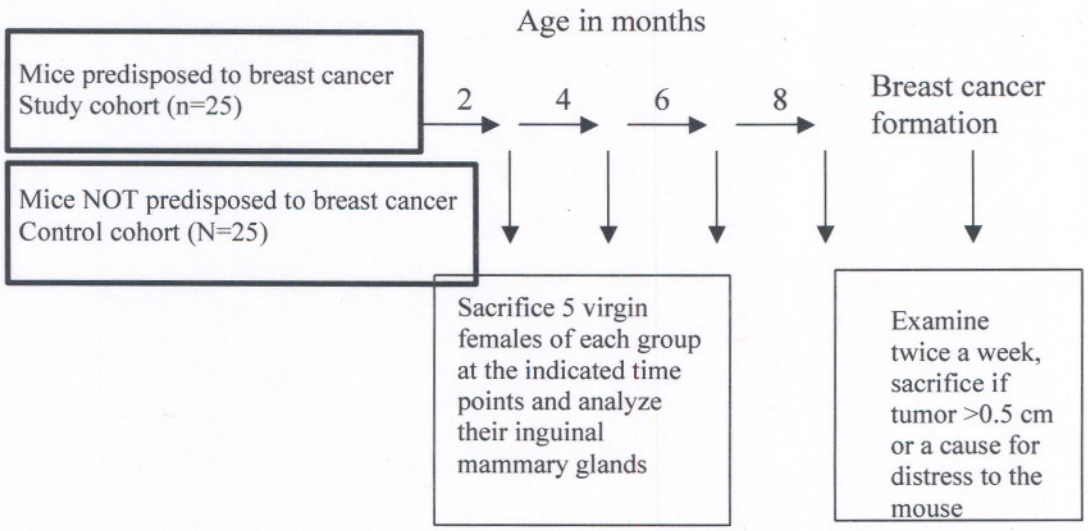
Fig. 1. Cancer-like colonies do not emerge in BRCA1-null PMECs (upper panel), but in BRCA1-null PMECs that are also p53 heterozygotes (lower panel). Left 4x, right 20x magnifications.

To indroduce p53 heterozygosity, founder animals were bought from Charles River, or obtained through the NCI repository. Members of the second generation (single transgene positive, and transgene negative) were used to breed a third generation in which 50% of the animals are transgene negative and 50% are transgene positive. This third generation, transgene positive study subjects, and transgene negative controls, are the study cohort. The transmission of the desired genotypes follows Mendelian genetics. Efficiency is 12.5 % or 1 in 8. For each mouse, genotyping will be performed at ages 2-6 weeks. The mice will be tagged with numbered eartags using . A less than 0.5 cm piece of the tail tip will be cut. Every effort will be made to have that tail tip be as short as possible to avoid pain and hemorrhage. These female virgin mice will be followed and sacrificed at the indicated time points or if they develop tumors (see flow chart). The mammary glands will be isolated, and the mammary epithelial cells analyzed as indicated above.

Breeding BRCA1 conditional knock-out with mutant p53 / cre



Flow Chart 1: Breeding schema, note that only 25 % of the bred female animals will be analyzed (red arrow)



Each experiment investigates 50 animals (25 study subjects, and 25 controls)

Flow Chart 2. Observation and disposition of the experimental cohorts

Research Accomplishments
1. Established colony of conditional BRCA1 mutant mice
2. Determined that cancer-like colonies can only be found if BRCA1-null primary mammary epithelial cells are also p53 heterozygote.

3. Established colony of conditional BRCA1 mutant mice with additional p53 heterozygosity
4. Developed ex vivo colony formation assay for these mice
5. Established a bank of fixed and frozen colonies that are available for tumor marker analyses.

Reportable Outcomes
The preparation of a publication of these data is in process.

Conclusions

Cancerization of PMECs is a complex process. In BRCA1 null mice, an additional genetic hit is required for PMECs to show features of cancerization in ex vivo cultures, i.e. heterozygosity for p53.

References

1. Venkitaraman, A.R., *Cancer susceptibility and the functions of BRCA1 and BRCA2.* Cell, 2002. **108**(2): p. 171-82.
2. Cavalli, L.R., et al., *Loss of heterozygosity in normal breast epithelial tissue and benign breast lesions in BRCA1/2 carriers with breast cancer.* Cancer Genet Cytogenet, 2004. **149**(1): p. 38-43.
3. Hoogerbrugge, N., et al., *High prevalence of premalignant lesions in prophylactically removed breasts from women at hereditary risk for breast cancer.* J Clin Oncol, 2003. **21**(1): p. 41-5.
4. Wulf, G., et al., *Modeling breast cancer in vivo and ex vivo reveals an essential role of Pin1 in tumorigenesis.* Embo J, 2004. **23**(16): p. 3397-407.

U.S. Army Medical Research and Materiel Command Animal Use Report

Facility Name: Beth Israel Deaconess Medical Center
Address: NRB 1030c, BIDMC, 330 Brookline Ave

Principal Investigator:

Principal Investigator: Gerburg M Wulf MD PhD
(Typed/Printed Name)

E-mail Address: gwulf@bidmc.harvard.edu

Contract Number:

Phone Number: 617 667 0843

Fax Number: 617 667 0610

This Report is for Fiscal Year (01 October - 30 September): 05/06

AAALAC* Accreditation Status (circle one): Full **x** Provisional Not Accredited Initial date of Accreditation (MM/YYYY) _03/05/2003__#A3431-01 Date of Most Recent Site Visit (MM/YYYY) ___05/19/2005___

Date of Last USDA Inspection: ___06/11/2007___ USDA Registration Number: _14-R-0138_____

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A. Animal	B. Number of animals purchased, bred, or housed but **not** yet used	C. Number of animals used involving no pain or distress	D. Number of animals used in which appropriate anesthetic, analgesic, or tranquilizing drugs were used to alleviate pain	E. Number of animals used in which pain or distress was not alleviated	F. Total Number of Animals (Columns C+D+E)
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Cats					
Guinea Pigs					
Hamsters					
Rabbits					
Non-human Primates					
Sheep					
Pigs					
Goats					
Horses					
Mice	90	70	0	0	160
Rats					
Fish					
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PURPOSE: The purpose of this form is to gather data related to animal use for research, development, testing, evaluation, clinical investigations, diagnostic procedures, and/or instructional programs conducted by or for the U.S. Army Medical Research and Materiel Command. This report is **NOT** to be used to report all animals used at your facility unless all work was conducted under contract with the U.S. Army Medical Research and Materiel Command. A separate form must be prepared for each award. This report is to cover one fiscal year (01 October - 30 September).

DEFINITIONS/INSTRUCTIONS:

Column A: List all animals used for the research, development, testing, evaluation, clinical investigations, diagnostic procedures, and/or instructional programs conducted. For the purpose of this report, an animal is defined as **any living nonhuman vertebrate**.

Column B: Refers to the purchase, breeding, or other acquisition of individual animals in the reporting fiscal year for assignment to a particular work unit or protocol. Animals carried over from the previous fiscal year and not yet used in any procedures or studies, must be included in this number for the work unit or protocol to which they are assigned.

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Column E: Number of animals used in painful procedures in which pain relieving compounds were not administered.

Column F: Sum of columns C, D, and E.

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Commander
U.S. Army Medical Research and Materiel CommandATTN: MCMR-RCQ-AR
504 Scott Street
Fort Detrick, MD 21702-5012

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1.a. NAME OF CONTRACTOR/SUBCONTRACTOR	c. CONTRACT NUMBER	2.a. NAME OF GOVERNMENT PRIME CONTRACTOR	c. CONTRACT NUMBER	3. TYPE OF REPORT (X one)
Wulf, Gerburg	BC 046324			☐ a. INTERIM ☑ b. FINAL

b. ADDRESS (Include ZIP Code)	d. AWARD DATE (YYYYMMDD)	b. ADDRESS (Include ZIP Code)	d. AWARD DATE (YYYYMMDD)	4. REPORTING PERIOD (YYYYMMDD)
				a. FROM
				b. TO

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME(S) OF INVENTOR(S) (Last, First, Middle Initial) a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS (X) d.				CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER (X) e.	
			(1) UNITED STATES		(2) FOREIGN			
			(a) YES	(b) NO	(a) YES	(b) NO	(a) YES	(b) NO
	None							

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) NAME OF INVENTOR (Last, First, Middle Initial)	(2) (a) NAME OF INVENTOR (Last, First, Middle Initial)	(1) TITLE OF INVENTION	(2) FOREIGN COUNTRIES OF PATENT APPLICATION
(b) NAME OF EMPLOYER	(b) NAME OF EMPLOYER		
(c) ADDRESS OF EMPLOYER (Include ZIP Code)	(c) ADDRESS OF EMPLOYER (Include ZIP Code)		

SECTION II - SUBCONTRACTS *(Containing a "Patent Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME OF SUBCONTRACTOR(S) a.	ADDRESS (Include ZIP Code) b.	SUBCONTRACT NUMBER(S) c.	FAR "PATENT RIGHTS" d.		DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S) e.	SUBCONTRACT DATES (YYYYMMDD) f.	
			(1) CLAUSE NUMBER	(2) DATE (YYYYMM)		(1) AWARD	(2) ESTIMATED COMPLETION
	None						

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR *(Not required if: (X as appropriate)* ☐ SMALL BUSINESS ☑ NON-PROFIT ORGANIZATION

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL (Last, First, Middle Initial)	b. TITLE	c. SIGNATURE	d. DATE SIGNED
Wulf, Gerburg M			11-01-07

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GENERAL

This form is for use in submitting INTERIM and FINAL invention reports to the Contracting Officer and for use in reporting the award of subcontracts containing a "Patent Rights" clause. If the form does not afford sufficient space, multiple forms may be used or plain sheets of paper with proper identification of information by item number may be attached.

An INTERIM report is due at least every 12 months from the date of contract award and shall include (a) a listing of "Subject Inventions" during the reporting period, (b) a certification of compliance with required invention identification and disclosure procedures together with a certification of reporting of all "Subject Inventions," and (c) any required information not previously reported on subcontracts containing a "Patent Rights" clause.

A FINAL report is due within 6 months if contractor is a small business firm or domestic nonprofit organization and within 3 months for all others after completion of the contract work and shall include (a) a listing of all "Subject Inventions" required by the contract to be reported, and (b) any required information not previously reported on subcontracts awarded during the course of or under the contract and containing a "Patent Rights" clause.

While the form may be used for simultaneously reporting inventions and subcontracts, it may also be used for reporting, promptly after award, subcontracts containing a "Patent Rights" clause.

Dates shall be entered where indicated in certain items on this form and shall be entered in six or eight digit numbers in the order of year and month (YYYYMM) or year, month and day (YYYYMMDD). Example: April 1999 should be entered as 199904 and April 15, 1999 should be entered as 19990415.

1.a. Self-explanatory.

1.b. Self-explanatory.

1.c. If "same" as Item 2.c., so state.

1.d. Self-explanatory.

2.a. If "same" as Item 1.a., so state.

2.b. Self-explanatory.

2.c. Procurement Instrument Identification (PII) number of contract (DFARS 204.7003).

2.d. through 5.e. Self-explanatory.

5.f. The name and address of the employer of each inventor not employed by the contractor or subcontractor is needed because the Government's rights in a reported invention may not be determined solely by the terms of the "Patent Rights" clause in the contract.

Example 1: If an invention is made by a Government employee assigned to work with a contractor, the Government rights in such an invention will be determined under Executive Order 10096.

Example 2: If an invention is made under a contract by joint inventors and one of the inventors is a Government employee, the Government's rights in such an inventor's interest in the invention will also be determined under Executive Order 10096, except where the contractor is a small business or nonprofit organization, in which case the provisions of 35 U.S.C. 202(e) will apply.

5.g.(1) Self-explanatory.

5.g.(2) Self-explanatory with the exception that the contractor or subcontractor shall indicate, if known at the time of this report, whether applications will be filed under either the Patent Cooperation Treaty (PCT) or the European Patent Convention (EPC). If such is known, the letters PCT or EPC shall be entered after each listed country.

6.a. Self-explanatory.

6.b. Self-explanatory.

6.c. Self-explanatory.

6.d. Patent Rights Clauses are located in FAR 52.227.

6.e. Self-explanatory.

6.f. Self-explanatory.

7. Certification not required by small business firms and domestic nonprofit organizations.

7.a. through 7.d. Self-explanatory.

BETH ISRAEL DEACONESS
MEDICAL CENTER
A member of CAREGROUP

A major teaching hospital
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The
Susan Komen Foundation

RE: Variance Statement for Financial Report BCTR061030, period 05/01/06-04/30/07

November 1, 2007

Dear Sir/Madam:

We were able to spend less money, specifically $ 8424.02 on salaries, through a cost sharing agreement with a different grant. The $ 8424.02 was instead spent on supplies that had gone up in price to an extent that was not foreseen at the time of the initial budget. There is no change in the goals, aims or scope of the work to be done.

Thank you for your attention to the matter,

Gerburg M. Wulf
Attending Physician
Assistant Professor – Harvard Medical School